Influence of acyl chain length on the enantioselectivity of *Candida antarctica* lipase B and its thermodynamic components in kinetic resolution of *sec*-alcohols

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Abstract

The enantioselectivity, *E*, of *Candida antarctica* lipase B (CALB) was found to be strongly influenced by the chain length of the achiral acyl donor employed in the transesterification of 3-methyl-2-butanol. Of the four studied acyl donors, the longest, vinyl octanoate, afforded the highest enantioselectivity. This was true over the temperature range studied, 6–70°C. Measurements of the temperature dependence of *E* allows for separation of the enthalpic and entropic components of enantioselectivity. Changes in *E* with chain length were mainly caused by changes in the entropic component except for the reaction with vinyl propionate, which differed from the others also in the enthalpic component. Optimisation of acyl donor adds one more possibility to improve the yield of enantiopurity in the production of optically active compounds apart from optimisation of solvent, temperature, water activity, and choice of enzyme. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

The lipase B from *Candida antarctica*, CALB, has been shown to be an excellent biocatalyst for kinetic resolution of *sec*-alcohols [1]. Its enantioselectivity is generally very high and it can withstand a great variation in experimental conditions [2]. Orrenius et al. proposed in 1998 a model for how CALB differentiates between enantiomers of *sec*-alcohols. The model involves two different orientation modes for the substrate in transition state during catalysis

and considers enthalpic energy differences between the enantiomers in their respective transition state [1]. Enantioselectivity, *E*, is not only the result of enthalpic energy differences between enantiomers but also contains an entropic component according to Eqs. 1 and 2 [3,4]:
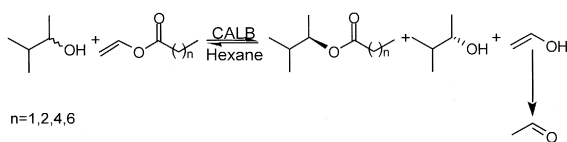
$$\Delta\Delta G^{\ddagger} = -RT\ln E, \tag{1}$$

$$\ln E = -\frac{\Delta\Delta H^{\ddagger}}{R}\frac{1}{T} + \frac{\Delta\Delta S^{\ddagger}}{R}. \tag{2}$$

By studying the temperature dependence of *E* the activation enthalpy and activation entropy difference between the enantiomers, $\Delta\Delta H^{\ddagger}$ and $\Delta\Delta S^{\ddagger}$, can be determined from the straight line of $\ln E$ vs. $1/T$. We have previously reported the entropic component

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Scheme 1.

of enantioselectivity, $T\Delta\Delta S^{\ddagger}$, at ambient temperature to be in the same order of magnitude as the enthalpic for some transesterification reactions of small *sec*-alcohols catalysed by CALB [5]. Previous reports have given indications that the length of the acyl chain would influence the success of the kinetic resolution of enantiomers of chiral alcohols catalysed by CALB [6,7]. The model of Orrenius et al. does not include acyl variation, which led us to investigate this further. In the present study we report how the enantioselectivity and its thermodynamic components in the transesterification reaction of 3-methyl-2-butanol, Scheme 1, depend on the length of the acyl chain of the achiral vinyl ester employed as acyl donor.

2. Experimental

2.1. Kinetic resolution of 3-methyl-2-butanol

25–200 mg of the CALB preparation Novozyme 435 from Novo Nordisk A/S (Denmark) was equilibrated in the reaction vessel to a water activity of 0.11 (LiCl (sat., aq.)) for more than 10 h. Dried (molecular sieves) 3-methyl-2-butanol (0.43 M) and hexane were added to the enzyme in the reaction vessel and equilibrated in temperature. The reaction was started by addition of vinyl ester (0.43 M). The vinyl esters used were vinyl octanoate, hexanoate, butanoate and propionate. Samples were taken with a syringe at regular intervals for conversions up to 50%.

2.2. Determination of enantioselectivity

The samples were analysed for the enantiomeric excess of remaining substrate 3-methyl-2-butanol, ee_s, and its produced ester, ee_p, with chiral capillary GC. All chiral compounds were analysed on the Chirasil-Dex CB column from Chrompack (The

Netherlands) except for 3-methyl-2-butyl propionate that was analysed on the Chiraldex G-PN column from Astec (USA). The enantiomeric ratio was determined as an average of 4–14 samples, typically 8, at conversions 0–50%, according to Rakels et al. [8] with the following equation:

$$E = \frac{\ln\left[\dfrac{1 - ee_s}{1 + ee_s/ee_p}\right]}{\ln\left[\dfrac{1 + ee_s}{1 + ee_s/ee_p}\right]}. \tag{3}$$

3. Results and discussion

It is intriguing to note that the length of the acyl chain strongly influences the success of the resolution of a chiral alcohol. The enantioselectivity of the reaction in Scheme 1 was strongly influenced by the acyl chain length at all temperatures studied (Fig. 1).

Vinyl octanoate yielded the most enantiopure product. Enantioselectivity decreased with decreasing chain length for vinyl hexanoate and vinyl butanoate. Shortening the acyl chain with one more carbon to vinyl propionate increased the enantioselectivity somewhat. Despite the fact that water activity changes with temperature, we do not believe this
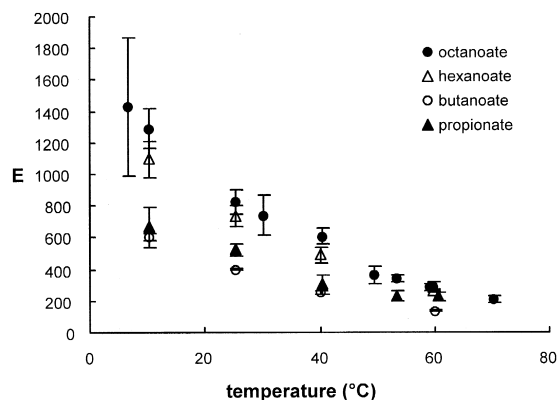

Fig. 1. E as a function of temperature for the transesterification of 3-methyl-2-butanol with vinyl octanoate, vinyl hexanoate, vinyl butanoate and vinyl propionate catalysed by *Candida antarctica* lipase B. Error bars show ± one standard deviation of the experimental data.

Table 1

Thermodynamic components of the enantioselectivity for the resolution of 3-methyl-2-butanol catalysed by CALB using different vinyl esters

Acyl donor	E (298 K)[a]	$\Delta\Delta G_{R-S}^{\ddagger}$ (298K) kJ/mole	$\Delta\Delta H_{R-S}^{\ddagger}$ kJ/mole	$\Delta\Delta S_{R-S}^{\ddagger}$ J/mole K	$T\Delta\Delta S^{\ddagger}$ (298 K) kJ/mole
Vinyl propionate	470	−15.3	−18.7 ± 1.9	−11.5 ± 6.1	−3.4 ± 1.8
Vinyl butanoate	390	−14.8	−23.6 ± 1.2	−29.6 ± 4.0	−8.8 ± 1.2
Vinyl hexanoate	720	−16.3	−22.3 ± 1.4	−20.1 ± 4.5	−6.0 ± 1.4
Vinyl octanoate	810	−16.6	−24.3 ± 1.1	−25.9 ± 3.4	−7.7 ± 1.0

[a] Calculated from the linear equation $\ln E$ vs. $1/T$.

to majorly affect the quality of the measured E-values. Our impression is that the enantioselectivity of CALB is not particularly dependent on water activity. As a matter of fact, Wehtje et al. [9] found no affect of water activity in the esterification of 2-octanol with decanoic acid. The respective contributions of thermodynamic components to E are listed in Table 1.

The longer acyl chains (C4, C6, and C8) differ mainly in the entropic component. For the propionate ester, both the enthalpic and the entropic component markedly differ from the other chain lengths. Estimation of the individual activation parameters, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$, indicated that the observed changes in the enthalpic and entropic component of E mainly lies on the S-enantiomer (data not shown). The estimation was done by assuming that the enantiomers have similar K_M allowing for separation of the total initial rate into the initial rate for each enantiomer by the relation $E = v_R/v_S$. Rate constants were calculated assuming saturating conditions and an enzyme load of 1.3 wt.% (Didier Rotticci, personal communication). Arrhenius plots ($\ln k_{cat} = -E_a/RT + \ln A$) then gives the activation parameters as $\Delta H^{\ddagger} = E_a - RT$ and $\Delta S^{\ddagger} = R\ln(AN_Ah/RT) - R$. No inactivation of the enzyme was observed after storage at 60°C for more than 19 h, a time interval well exceeding any of the reaction times.

CALB has a deep narrow active site into which the substrate ester binds in a hairpin structure [10]. The acyl and alcohol moieties are thereby brought close in space during catalysis, which could explain CALB's sensitivity to the length of the acyl chain. In a molecular modelling study on the enantioselectivity of CALB towards sec-alcohols, Hæffner et al. [11] observed that amino acid residues in the acyl binding site affected the enantioselectivity. Not only interactions calculated between the chiral substrate and amino acid residues in the vicinity of the point of chirality were found to contribute to E, but also interactions with residues in the acyl binding site at some distance from the chiral carbon. In further molecular modelling studies on CALB enantioselectivity, focusing on the influence of the acyl chain length, it was noted that the acyl chain of the propionate ester had a different position compared to other acyl chains [Sami Raza, personal communication]. Instead of overlapping with the first three carbons of the long chain of Tween 80 in its crystal structure with CALB, as longer acyl chains do, the propionate chain can point directly out towards the protein surface [10]. Possibly, this additional position of the acyl chain could be the cause of the changes in thermodynamic components of the enantioselectivity observed for vinyl propionate in the present investigation.

In conclusion, we note that the choice of achiral acyl donor strongly influences the success of resolving a chiral alcohol in transesterification reactions with CALB. Choosing a longer acyl donor increased the enantiopurity of the final product. This adds yet another means of optimising the enantiomeric yield for the production of optically active compounds apart from optimisation of solvent, temperature, water activity and choice of enzyme.

Acknowledgements

References

[1] C. Orrenius, F. Hæffner, D. Rotticci, N. Öhrner, T. Norin, K. Hult, Biocatal. Biotransform. 16 (1998) 1–15.

[2] E.M. Anderson, K.M. Larsson, O. Kirk, Biocatal. Biotransform. 16 (1998) 181–204.

[3] R.S. Phillips, Enzyme Microb. Technol. 14 (1992) 417–419.

[4] R.S. Phillips, Trends Biotechnol. 14 (1996) 13–16.

[5] P.L.A. Overbeeke, J. Ottosson, K. Hult, J.A. Jongejan, J.A. Duine, Biocatal. Biotransform. 17 (1999) 61–79.

[6] M. Takeda, T. Ishizuka, K. Itoh, T. Kitazume, J. Fluorine Chem. 75 (1995) 111–113.

[7] R. Morrone, G. Nicolosi, A. Patti, Gazz. Chim. Ital. 127 (1997) 5–9.

[8] J.L.L. Rakels, A.J.J. Straathof, J.J. Heijnen, Enzyme Microb. Technol. 15 (1993) 1051–1056.

[9] E. Wehtje, D. Costes, P. Adlercreutz, J. Mol. Catal. B: Enzym. 3 (1997) 221–230.

[10] J. Uppenberg, N. Öhrner, M. Norin, K. Hult, G.J. Kleywegt, S. Patkar, V. Waagen, T. Anthonsen, T.A. Jones, Biochemistry 34 (1995) 16838–16851.

[11] F. Haeffner, T. Norin, K. Hult, Biophys. J. 74 (1998) 1251–1262.